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Development of a Monolithic HPLC-ECD Method for the Determination of Ropinirole HCl in Mouse Sera and Dosage Forms

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Authors' contributions

This work was carried out in collaboration between all authors. Author MR designed the study. Author MH wrote the protocol. Author RTEE managed the literature searches. Authors AAM, MM and SA performed the analysis, managed the experimental process and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: A highly selective, sensitive, and rapid to establish an innovative way to determine Ropinirole HCl in mouse sera and pharmaceutical formulations.
Study Design: High-performance liquid chromatography (HPLC) method with electrochemical detector (ECD) was developed.
Place and Duration of Study: College of Pharmacy, King Saud University, between May 2015 and December 2015.

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Methodology: The chromatographic separation was achieved on a reversed phase RP-18e Chromolith Performance column (100 mm × 4.6 mm) with a mobile phase of methanol: 50 mM sodium dihydrogen phosphate (pH 4.5) (10:90, v/v) pumped at a flow rate of 2.0 mLmin^{-1}. Paracetamol was used as an internal standard (IS). Ropinirole HCl and the IS were extracted from mouse sera by using the deproteinisation procedure, followed by injection of an aliquot of the supernatant into the chromatographic system. The separation of the studied drugs was achieved within 3 min.

Results: The proposed HPLC-ECD method was validated for its selectivity, linearity, accuracy, precision, robustness and stability. The calibration curves in serum showed excellent linearity (r = 0.9980) over concentrations ranging from 10 to 2400 ng mL^{-1} for ROP with limit of detection (LOD) equal to 2.5 ng mL^{-1} which is lower than those obtained with a UV-VIS detector.

Conclusion: The method was successfully utilised for Ropinirole HCl quantification in mouse sera samples and good recoveries were obtained without interference from endogenous uric acid and dopamine. Moreover, the assay was successfully applied in a pharmacokinetic study. In addition, the proposed HPLC-ECD method was applied in the determination of ROP content in tablet dosage forms, with good recoveries were obtained without interference from their excipients; Na^+, K^+, Ca^{2+}, Mg^{2+}, lactate, sucrose, lactose and starch.

Keywords: High performance liquid chromatography; electrochemical detection; monolithic column; Ropinirole; mouse sera.

1. INTRODUCTION

Ropinirole hydrochloride (ROP), 4-[2-(dipropylamino) ethyl]-l, 3-dihydro-2H-indol-2-one hydrochloride, (Fig. 1) [1], is a novel nonergoline dopamine D_2-receptor agonist, indicated for the treatment of early and advanced Parkinson's disease. It is used as monotherapy in the first stages of the disease [2]. ROP is one of the four drugs approved by the FDA for the treatment of primary restless legs syndrome affecting up to ten percent of the population [3]. High-performance liquid chromatography (HPLC) is one of the most widely used techniques for quantitative analysis in complicated matrices [4-7]. Electrochemical detector (ECD) provides high sensitivity and specificity for substances that are either oxidized or reduced at the applied potential. HPLC coupled with electrochemical detector (HPLC-ECD) provides an analytical tool for resolving and accurately detecting trace amounts of electroactive compounds in biological fluids [8]. A search of the scientific literature indicates that, several analytical methods have been described for the determination of ROP, such as spectrophotometry [9], spectrofluorimetry [10], voltammetry [11,12], high performance thin layer chromatography [13], HPLC with UV detection for drug impurity profiling [14,15] and stability-indicating assays

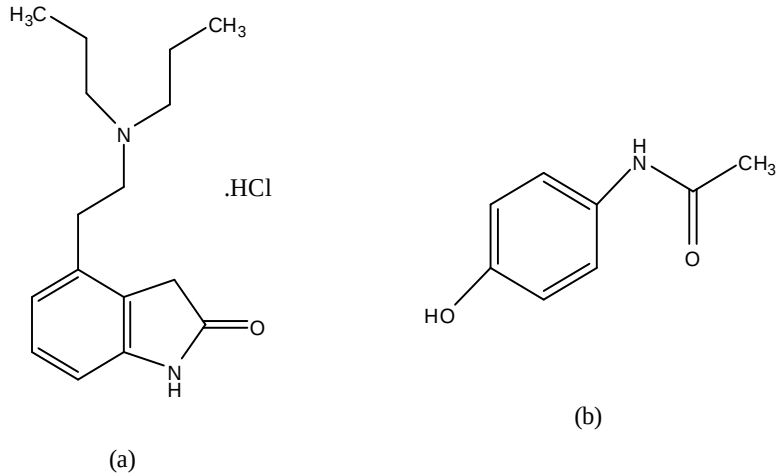


Fig. 1. Chemical structure of (a) ROP and (b) Paracetamol (IS)

[16], ultra-performance liquid chromatography [17,18], liquid chromatography-mass spectrometry [19,20] and capillary zone electrophoresis [21]. The official method for quantification of ROP has been approved in USP, where HPLC-UV method was recommended [22]. In addition to sensitivity, run times are an important aspect of chromatographic methods. The total analysis times of published HPLC-UV methods for ROP are often longer than 10 min [14,15]. In LC-MS methods, the run times were within 1.5 min [19,20], but LC-MS associated with major drawbacks via employed an expensive tandam mass detector that is not available in most laboratories. In recent years, the monolithic stationary phases as separation media for HPLC have undergone a rapid development in the field of sample analysis because of its excellent performance, such as fast mass transport and highly permeability [23]. Monolithic column offer increased chromatographic efficiency, resolution and shorter analysis times [24]. Up to date, no analytical method has been published concerning the analysis of ROP combining the sensitivity of HPLC–ECD with the superior separation and speed of a monolithic column. Therefore, this work aimed to develop a rapid and sensitive monolithic column HPLC–ECD method for the analysis of ROP in biological fluids and formulations and to validate it according to the ICH guidelines for bioanalytical method validation [25].

2. EXPERIMENTAL

2.1 Reagents and Materials

ROP (> 99% purity)is supplied from (RA CHEM Pharm Ltd, B.N. RPN/WS/001/12) as a reference material and is used as such, paracetamol (> 99% purity) as an IS were purchased from Sigma Chemical (St. Louis, MO, USA).Tremodect® film-coated tablets (Eva Pharma, Kafr El Gabal, Giza, Egypt) containing 4.56 mg of ROP, equivalent to 4 mg of Ropinirole, was supplied from local pharmacies. HPLC-grade methanol, acetonitrile and analytical grade sodium dihydrogen phosphate, diethylamine and *ortho*-phosphoric acid were obtained from BDH Chemicals (City, UK). Bidistilled water was purified using a cartridge system (Milford, USA). Ultrapure water with a sensitivity of 18 µΩ was obtained from a Millipore Milli-Q plus purification system, Waters Milford, USA. Adult male Swiss albino mice were provided by the Experimental Animal Care Centre at the college of Pharmacy, KSU.

2.2 Instrumentation and Chromatographic Conditions

The HPLC analysis was performed on a Waters HPLC System (Milford, MA, USA), equipped with a 1500 series HPLC pump and an autosampler (2707). Detection was carried out using an Electrochemical Detector (Antec Decade, DB Leiden, the Netherlands) equipped with a glass carbon electrode (working electrode) and an Ag/AgCl electrode (reference electrode). The data were processed by means of an Empower Pro Chromatography Manager Software (Waters Corporation). All solutions were filtered through a 0.22 µm Millex filter (EMD Millipore, Milford, MA, USA) and degassed by ultra sonication (Tecnal, São Paulo, Brazil). Chromatographic separation was achieved on a reversed-phase RP-18e Chromolith Performance column (100 mm × 4.6mm; Merck, Darmstadt, Germany), which was coupled to a Water Symmetry C18 Sentry Guard column (20 mm). The mobile phase consisted of methanol: Sodiumdihydrogen phosphate (50 mM) (10: 90, v/v). The pH of the mobile phase was adjusted with diethyl amine to pH = 4.5. The mobile phase was freshly prepared, then filtered and degassed. All resolutions were performed isocratically at a flow rate of 2 mL min^{-1}. The injection volume was 20 µL, and the chromatograms were monitored by an electrochemical detector set at (V =+1.2V). Column temperature was maintained at room temperature (25 ± 2℃).

2.3 Preparation of Stock and Standard Solutions

Primary stock solutions of ROP were prepared for use as standards and quality controls (QC) by dissolving an accurately weighed amount of ROP in deionized water and diluting to obtain a concentration of 1.0 mg mL^{-1}. An internal standard stock solution was prepared by dissolving an accurately weighed amount of paracetamol and diluting in methanol to obtain a concentration of 1.0 mg mL^{-1}. Working standard solutions of ROP and IS were prepared in deionized water to get the working solution of 10.0 µg mL^{-1}. All solutions were found to be stable for two months when stored at -4℃. Appropriate dilutions of the working solution of ROP were made and used for constructing the

calibration curves and spiking serum. All solutions contained the internal standard at a concentration of 100 ng mL^{-1}.

2.4 Preparation of Serum Quality Control Samples

The quality control (QC) samples at five concentration levels, i.e. 35, 215, 720, 1680, 2160 ng mL^{-1} for ROP were prepared by spiking the drug-free serum with appropriate volumes of ROP and the internal standard at a concentration of 100 ng mL^{-1} and were stored frozen until analysis. Before spiking, the drug free serum was tested to make sure that there was no endogenous interference at a retention time of ROP and the internal standard. The QC samples were extracted with the calibration standards as under (section 2.6.) and the percentage biological content of the stored QC samples was found in the accepted range (98–101.5%).

2.5 Assay Method

A mouse sera sample (50 µL) was placed in 1.5 mL Eppendorf tubes, and accurately measured aliquots of the working standard ROP (10.0 µg mL^{-1}) were added. Then 10 µL of the diluted internal standard solution (5 µg mL^{-1}) was added to each tube and diluted to 500 µL with deionized water and mixed well to give final concentrations of 35, 215, 720, 1680, 2160 ng mL^{-1} for ROP. The mixture was treated with 500 µL of acetonitrile for deproteinisation. Each tube was, subsequently, vortexed at high speed for 60 s, and centrifuged at 12000 rpm for 10 min. The supernatants were loaded into the autosampler tray and 20 µL of the supernatant was injected into the HPLC system in triplicate runs. Blank mice sera samples were processed in the same manner using deionized water instead of ROP.

2.6 Calibration Curves, Limit of Detection and Quantification

Calibration plots for the ROP in serum were prepared daily by diluting working solutions with pooled mouse sera to yield eight concentrations level, i.e. 10, 50, 100, 500, 1000, 1500, 2000 and 2400 ng mL^{-1}including the LOQ. Calibration standards at each concentration were treated as above (section 2.5) and analysed in six replicates. Calibration curves of ROP were constructed using the observed analyte peak area over the internal standard peak area versus nominal concentrations of the analytes. Least-squares linear regression analysis of the data gave slope, intercept and correlation coefficient data. From this data, a first order polynomial model was selected for ROP. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as 3 or 2 and 10 times the noise level, respectively, following by the ICH guidelines [25]. The results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear squares treatment of the results along with standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals ($S_{y/x}$), were obtained.

2.7 Precision and Accuracy

The intra-day and inter-day accuracy and precision of the assays in serum were determined by assaying five QC samples (35, 215, 720, 1680, 2160 ng mL^{-1}) for ROP in six replicates over a period of 3 days. The concentrations represented the entire range of the calibration curves. Calibration curves were prepared and analysed daily and linear models were used to determine concentrations in the QC samples. Precision was reported as a %relative standard deviation (%RSD) = (SD/mean) × 100. Percent accuracy was determined (using the data from the precision assessment) as the closeness of spiked samples to the nominal value of in-house standards. Percent accuracy was reported as %Er = (measured−nominal) /nominal × 100.

2.8 Animals

Adult male Swiss albino mice aged 10-14 weeks and weighing 25–30 g were obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University. Animals were acclimated in laboratory for 2 days under standard conditions of humidity, temperature (25 ± 2℃) and light (12 h light/12 h dark). Mice were fed with a standard mouse pellet diet and had free access to water. All animal procedures were in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH) protocol. The study protocol was approved by the Animal Ethics Committee of the Pharmacology Department, College of Pharmacy, King Saud University, Kingdom of Saudi Arabia. After 2 days of accommodation,

the mice were randomly divided into 10 groups, each treatment and control group consisted of 6 assigned mice.

2.9 Ropinirole Treatment and Serum Sampling

ROP was dissolved in saline and administered by gavage at a single dose level of 20 $mg \cdot kg^{-1}$ to nine groups and the remaining group of mice was administered saline to provide the blank mice plasma. The ingested volume was 0.01 $mL \cdot g^{-1}$ body weight. Blood samples from animals treated with Ropinirole were withdrawn by heart puncture after sacrificing groups of mice at 0.5, 1, 1.5, 2, 3, 4, 6, 20 and 24 h after drug exposure. At specific time, blood samples were collected from each group then the serum samples were separated from blood by centrifugation (3000 rpm at 4℃) for 10 min, and then stored at -20℃ until analysis.

2.10 Preparation of Tablet Solutions

Ten tablets of Tremodect® film-coated tablets were weighed and powdered in a mortar. An adequate amount of prepared powders, equivalent to 10 mg ROP was weighed and transferred to 100 mL volumetric flask diluted to the mark with purified water. The mixtures were sonicated for 15 min to provide complete dissolution of active ingredients and centrifuged at 3000 rpm for 10 min. Accurately measured aliquots of the supernatant were transferred to 10 mL volumetric flasks containing 100 µL of the internal standard and diluted to 10 mL with purified water to give final concentrations of 180, 320 and 800 ng mL^{-1} of ROP.

3. RESULTS AND DISCUSSION

3.1 Optimization of the Chromatographic Method

In order to optimize the sensitivity, selectivity and analysis time of our method for ROP and paracetamol (IS), chromatographic conditions, including mobile phase composition, salt type and concentration, flow rate, column type, as well as working potentials applied on the electrochemical detector were varied. To achieve optimum electron transfer in an electrochemical detection, a mobile phase with an electrolyte concentration between 50 and 100 mM L^{-1} is recommended. To ensure a sufficient ionic conductivity of the mobile phase for electrochemical detection, the aqueous part of mobile phase contained 50 mM L^{-1} electrolyte concentration was chosen. The salt type with trimethylamine/ phosphoric acid, lithium perchlorate/ perchloric acid, sodium acetate/ acetic acid, sodium dihydrogenphosphate/ phosphoric acid (NaH_2PO_4), disodium hydrogen phosphate/phosphoric acid were evaluated. Sodium dihydrogenphosphate, at a concentration of 50 mM L^{-1} and adjustment of the pH to 4.5 with phosphoric acid resulted in better peak shape. High salt concentrations may be problematic for the HPLC system and salt crystals were frequently observed in our system, which therefore had to be regularly washed with different solvents. The effect of the pH of NaH_2PO_4 buffer compatible with the column pH range of stability (from 3.0 to 7.0) was also tested. Well separated peaks were obtained at pH from 4.0 to 7.0. In addition, the efficiency of the chromatographic system expressed in terms of the number of the theoretical plates was maximum with pH 4.5. Trials were carried out with different volumes of acetonitrile and methanol in the mobile phase to improve the resolution of the analytes. The use of acetonitrile instead of methanol as organic solvent resulted in that complete separation of ROP from IS could not be achieved with the mobile phase unless consisting of up to 40% acetonitrile.The best peak shape and optimum resolution were obtained with the given mobile phase composition, which uses methanol as an organic modifier in low percentages (10%). The total chromatographic run time, was 3.0 min, which is shorter than that of HPLC-UV methods of ROP [14,15]. Experiments were also performed with RP-18e Chromolith column and different types of conventional stationary phase's fused core (Kinetex PFPand Kinetex C18) columns from Phenomenex, (Gemini and Luna C18). The best chromatogram with good peak symmetry and reproducibility was obtained using a RP-18e Chromolith column.

3.2 Method Validation

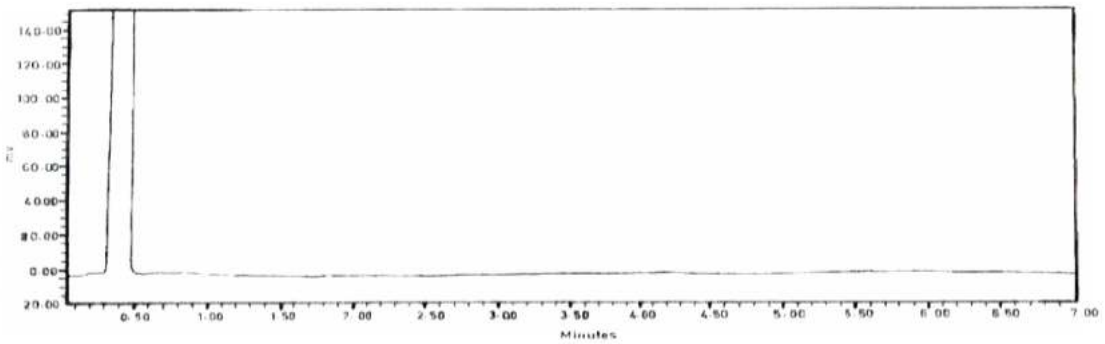
3.2.1 Selectivity of the method

The selectivity of a newly developed HPLC-ECD method for quantification of ROP was evaluated. The addition of possible interference compounds commonly present in the analysed pharmaceutical dosage forms or biological samples was investigated in a standard solution containing ROP at 200 ng mL^{-1} concentration. According to the analysis of the obtained

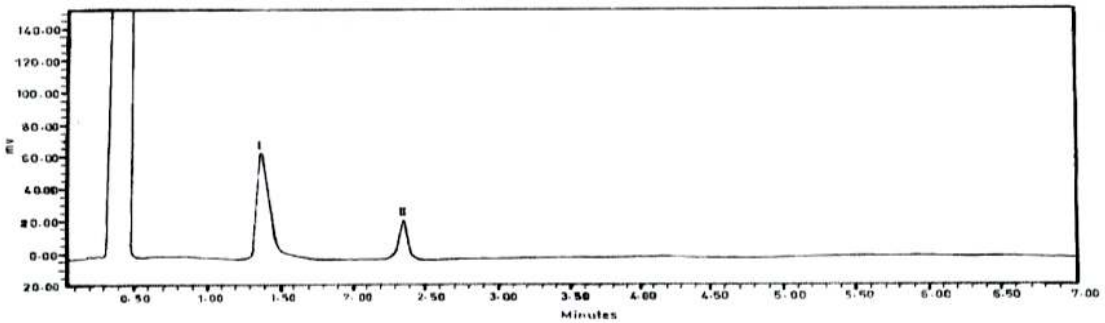
chromatogram responses, Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+} lactate, sucrose, lactose and starch (200-fold) did not show interference in the determination of ROP and IS. Also, the interference of glucose and uric acid was examined because these endogenous substances are always present in biological fluids. Moreover, blank serum samples obtained from 6 different sources were assayed to evaluate the selectivity of the developed HPLC-ECD method and the detection of interference. Representative chromatograms of mouse sera taken from mice after 3 h of administration of 20 mg kg^{-1} of ROP are shown in (Fig. 2). The peaks of ROP and the IS were well resolved, with retention times of 2.38 and 1.41 min, respectively. No endogenous peak of serum was found to interfere with the elution of ROP or the IS. Analysis was achieved within 3 min for a total chromatography run. The peaks were completely resolved one from another. This method enabled the accurate analysis of ROP in spiked, as well as in experimental mice serum (Fig. 3a). The proposed method also offers high sensitivity considering that 10 ng mL^{-1} of ROP could be determined accurately (Fig. 3b).

3.2.2 Linearity, Lower Limit of Detection (LOD) and Lower Limit of Quantification (LOQ)

The linearity of the detector response for the ROP in mouse serum was evaluated by triplicate injection of standard solutions corresponding to each point on the standard curve on 3 different days. The detector responses for ROP were linear from concentrations in the low 10 ng mL^{-1} range and up to 2400 ng mL^{-1} and regression coefficients (r) for all compounds were higher than 0.999 (Table 1). The limit of detection (LOD) and the limit of quantification (LOQ) were determined as recommended by the ICH guidelines [25]. The LOQ and LOD of ROP in mouse serum were 10 and 2.5 $ngmL^{-1}$, respectively (Table 1). Overall, our data indicate that HPLC-ECD is a highly sensitive method for the quantification of ROP in biological fluids and the possibility of evaluating clinical samples from patients under ROP treatment.

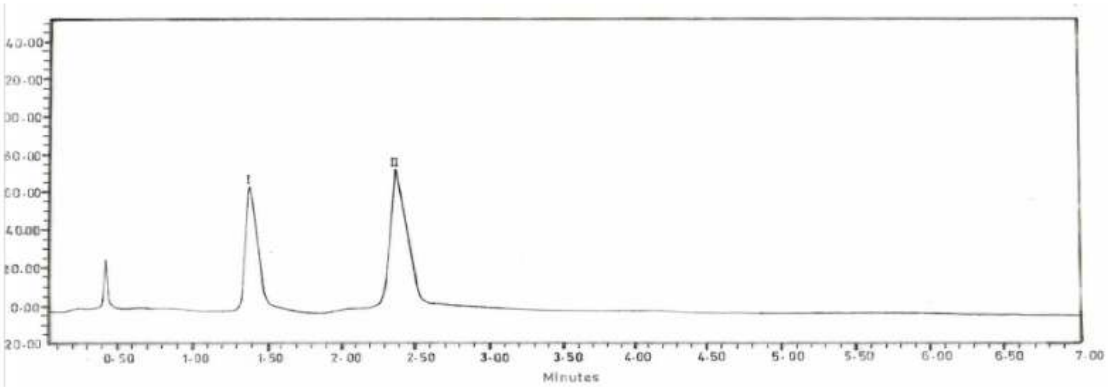


(a)

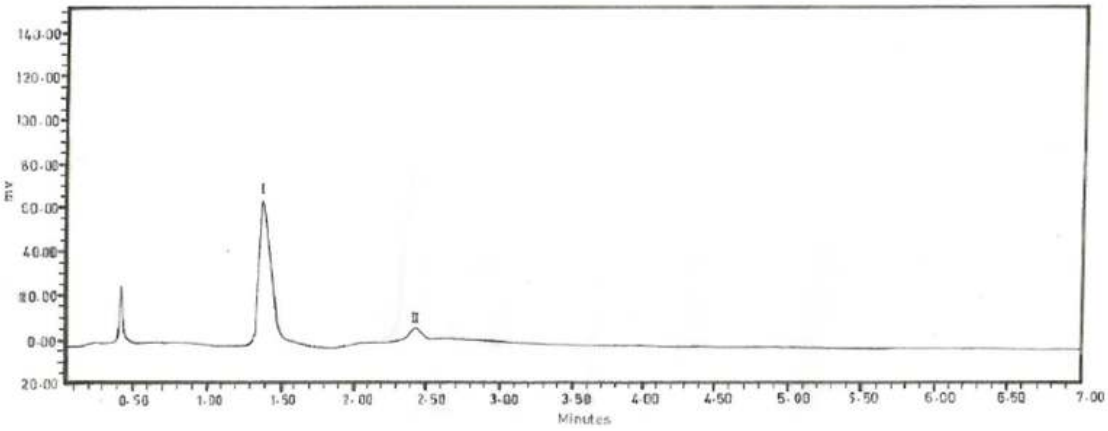


(b)

Fig. 2. Chromatograms of (a) blank mice serum and (b) serum taken from a mouse after 3 h administration of 20 mg·kg^{-1} ROP(II) and IS (I)



(a)



(b)

Fig. 3. Chromatograms of (a) obtained from spiked mice serum of (I) 100 ng mL^{-1}of paracetamol(IS) and (II) 100 ng mL^{-1}of ROP, and (b) LLOQ for ROP

3.2.3 Precision and accuracy

The precision and accuracy of the developed HPLC-ECD method were determined by using mouse sera samples spiked at five levels (Table 2). The data indicate that intra-day precision and accuracy (n = 6) as expressed by %RSD and % Er were 1.25 to 2.28% and -0.48 to 1.29%, for ROP, respectively. The inter-day precision and accuracy (n = 6) expressed by %RSD and %Er were 1.38 to 2.11% and 0-.7 to 2.43% for ROP, respectively. The results presented in (Table 2) indicated that the overall recovery of ROP from the spiked mouse serum ranged from 97.0 to 102.4%. The repeatability of retention times and peak areas were also investigated. Because many factors can affect the repeatability of the retention time in LC. The standard deviation values of the retention time of ROP and the IS for 6 replicates were 2.38 (± 0.15) and 1.51 (± 0.27), respectively, demonstrating that acceptable repeatability was attainable.

To test the accuracy of the proposed method, it was applied to the determination of ROP in bulk in different concentrations within the concentration range. The results obtained are in good agreement with those obtained using the official refrence method [22]. Using Student t-test and F-test revealed no significant differences between the performance of the two methods regarding accuracy and precision. The results are shown in (Table 3).

3.2.4 Stability

Quality-control samples of ROP were used for experiments designed to study the stability of the drug under various conditions. The deviation of the mean test responses was within ± 15% of

appropriate controls for ROP. No evidence of degradation was observed during sample processing and storage for at least 1 month in a -20℃ freezer. No effects on the quantification of ROP was observed after storing samples at room temperature for 6 h. The results of these studies suggest that mouse serum samples containing ROP can be handled under normal laboratory conditions, without significant degradation of the compound.

Table 1. Linear ranges and the validation parameters of the experimental data of ROP in spiked mice serum

Parameters	ROP
Concentration range (ng mL^{-1})	10 - 2400
Intercept (a)	0.8×10^{-3}
Slope (b)	1.1×10^{-3}
Correlation coefficient (r)	0.9994
$S_{Y/X}$	1.6×10^{-5}
S_a	1.3×10^{-3}
S_b	4.8×10^{-3}
LOD (ng mL^{-1})[a]	2.5
LOQ (ng mL^{-1})[b]	10
Repeatability (RDS, %)[c]	0.98

[a] *S/N = 3;* [b] *S/N = 10*

[c] *Expressed as % RSD = (S.D. / Mean) x 100*

S_a standard deviation of the intercept on the ordinate

S_b standard deviation of the slope on the ordinate

$S_{y/x}$ standard deviation of the residuals

3.2.5 Assay robustness and ruggedness

The method robustness was assessed as a function of the one-variable-at-a-time (OVAT) approach at 3 levels (0; ± 10%). Robustness was statistically analysed using peak area, retention time, and resolution of ROP and paracetamol as the IS. The method showed acceptable robustness levels using the OVAT approach, in terms of the retention time and peak area of each analyte. The OVAT procedure varies the levels of a given parameter, while keeping the other factors at nominal (optimal) levels. The results obtained after varying one parameter are then compared to those from experiments conducted with all parameters at optimal levels. The optimum HPLC-ECD conditions set for the developed methods were slightly modified to evaluate the robustness. The effects of different working potentials applied to the electrochemical detector (± 1.0%), different mobile phase composition (± 1.0%), buffer pH (± 0.2 units) and flow rate (± 10.0%), were determined. The results of robustness for different parameters are expressed as %RSD (n = 6), calculated as the standard deviation of the result at the investigated level and result at the zero level divided by their mean value. No significant variations in accuracy and precision (retention time, peak area) were found in the tested range, which indicates the efficient robustness of the method (RSD was 1.6%).

To evaluate the ruggedness of the HPLC-ECD method we performed the analysis using different instruments, two different analysts (operators) and on different days. The results obtained using two different analysts and different instruments were found to be reproducible. RSD values of less than 2.8% were observed for repetitive measurements and operators. These results indicate that the method is capable of producing results with high precision.

Table 2. Intra-day and inter-day reproducibility of quality control samples of ROP in spiked mice serum obtained by HPLC-ECD

Analyte	Actual Conc. (ng mL^{-1})	Experimental (ng mL^{-1})	Recovery (%)	RSD (%) [a]	Er (%) [b]
Intra-day [c]	35	33.98 ± 0.75	97.08	2.21	-2.92
	215	210.78 ± 3.20	98.03	1.52	-1.96
	720	729.37 ± 9.14	101.29	1.25	1.29
	1680	1655.31 ± 35.87	98.53	2.16	-1.47
	2160	2149.61 ± 49.12	99.52	2.28	-0.48
Inter-day [d]	35	33.69 ± .69	96.25	2.04	-3.74
	215	208.65 ± 2.87	97.04	1.38	-2.96
	720	737.54 ± 10.36	102.43	1.40	2.43
	1680	1666.56 ± 25.13	99.2	1.51	-0.80
	2160	2144.96 ± 45.36	99.3	2.11	-0.70

[a] *Expressed as % RSD: (SD/mean) x 100.*

[b] *Calculated as [(found conc. – actual conc)/ actual conc.] x100.*

[c] *Mean ± SD based on n = 6.*

[d] *Mean ± SD based on n = 6*

Table 3. Accuracy of the proposed HPLC-ECD method for the determination of ROP in bulk

Parameter	Proposed method	Reference method [22]
ROP Concentration ng mL^{-1}	**% Recovery**	**% Recovery**
215	98.03	99.78
720	101.29	98.86
1680	98.53	99.92
Average, %	99.28	99.52
SD	1.76	0.58
t-test	0.30 (2.78)*	
F test	11.49 (19.00)*	

**t tabulated and F tabulated values at (P =0.05)*

3.2.6 Pharmacokinetics study

The described method was further applied to a preliminary pharmacokinetics study of ROP in mice. The concentration of ROP in mice serum at different time interval after dosing was determined individually. After the determination, a serum concentration-time curve (AUC) for ROP were constructed, (Fig. 4). The main pharmacokinetic parameters of ROP was calculated from the curve. After drug administration, the mean value of T_{max} and C_{max} were 3 h and 215.98 ± 31.21 ng mL^{-1}, respectively.

3.2.7 Application to pharmaceutical formulations

To evaluate the validity and practical utility of the proposed method for quantification of ROP in commercially available pharmaceutical products (Tremodect® film-coated tablets, Eva Pharma, Kafr El Gabal, Giza, Egypt) were analysed in order to eliminate matrix effects. The results summarized in (Table 4) show that the content of assayed tablets obtained by utilizing the HPLC-ECD are in good agreement with those determined using HPLC reference method [22]. The effect of excipients (Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+} lactate, sucrose, lactose and starch) upon the electrochemical response of ROP at the

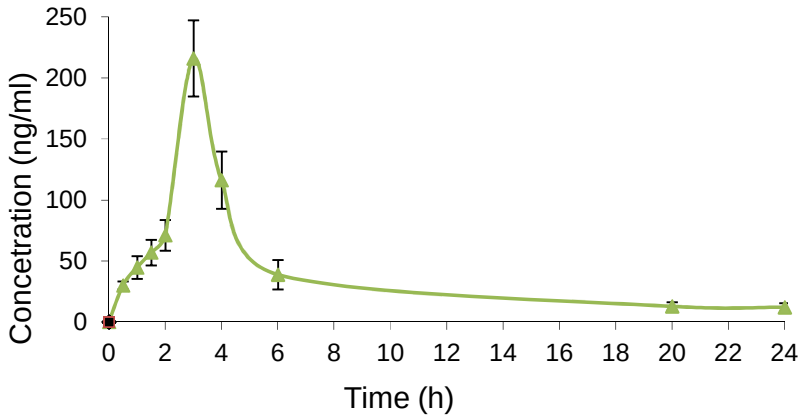


Fig. 4. Concentration-time profile of ROP in mice plasma after administration of ROP (20 mg·kg^{-1}). Each point represents the mean ± S.D of six mice

Table 4. Application of the proposed HPLC-ECD method for the determination of ROP in dosage forms and compared with the HPLC official method

Pharmaceutical preparation	Actual Conc. (ng mL^{-1})	Experimental (ng mL^{-1})	Recovery (%)	Official HPLC method [22]
Tremodect® film-coated tablets*	180	184.11	102.28	103.06
	320	325.76	101.80	105.75
	800	791.21	98.90	105.55
Overall recovery, %			100.99	104.79
SD			1.83	1.50
t Calculated				2.77 (2.78)**
F Calculated				1.43 (19)**

**Product of EvaPharma, Kafr El-Gabal, Giza, Egypt*
***t tabulated and F tabulated values at (P =0.05)*

a glassy carbon electrode was studied The recoveries of 101.01% for ROP were obtained from this type of matrix, indicating adequate accuracy of the proposed analysis procedures. The obtained recoveries revealed that excipients did not interfere with the assay, and thus corroborating the suitability of the proposed HPLC-ECD for this purpose.

4. CONCLUSION

A specific and rapid HPLC-ECD method with a monolithic column was developed for the determination of ROP in mouse sera and pharmaceutical products. The present investigation confirms the possibility of using monolithic columns with electrochemical detector to provide high specificity and separation efficiency that is comparable to more conventional reversed-phase HPLC systems.The proposed method was successful in eluting ROP with retention time of 2.38 min which is a nearly 3-fold reduction in the analysis time over published HPLC-UV/DAD methods, while operating at a back pressure that can be handled by most conventional HPLC systems. Thus, our method has the advantage of being compatible with standard HPLC pumps and the use of an electrochemical detector, which is less expensive in acquisition and maintenance than a mass detector, further improves its wide applicability and usefulness. The proposed method had LOD and LOQ of 2.5 and 10.0 ng mL^{-1} for ROP. Therefore, the developed method may be an alternative to classical HPLC-UV/DAD methods when samples with low ROP content are under investigation. The method was fully validated in accordance with ICHguidelines and the obtained results are within their acceptance criteria.

CONSENT

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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